

Atty. Dkt. No. 081356-0111

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:

Susmu KAJIWARA et al.

Title:

A DNA CHAIN USEFUL FOR INCREASING PRODUCTION OF

CAROTENOIDS

AUG 1 1 2003

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Appl. No.:

08/737,319

Filing Date:

11/12/1996

Examiner:

K. Kerr

Art Unit:

1652

TRANSMITTAL OF FORMAL DRAWINGS

Commissioner for Patents PO Box 1450 Alexandria, Virginia 22313-1450

ATTENTION: DRAWING REVIEW BRANCH

Sir:

Transmitted herewith are the formal drawings (14 sheets, Figures 1-14) for the above-identified application. The Official Draftsperson is respectfully requested to approve these drawings for entry into the application.

Respectfully submitted,

Stephen A. Bent

Attorney for Applicant Registration No. 29,768

Date August 7, 2003

FOLEY & LARDNER Customer Number: 22428

22428

22428

PATENT TRADEMARK OFFICE

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(202) 672-5404

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Title: A DNA CHAIN USEFUL FOR INCREASING PRODUCTION OF CAROTENOIDS

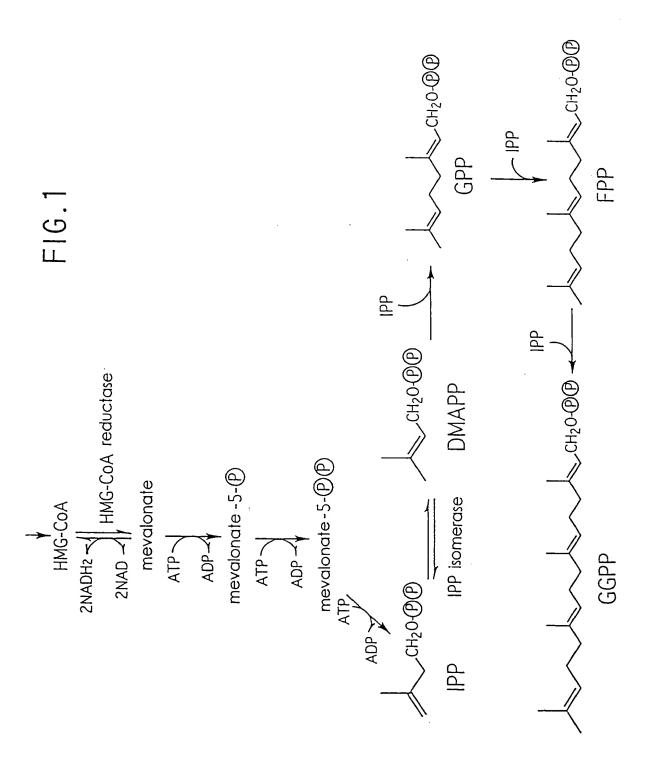
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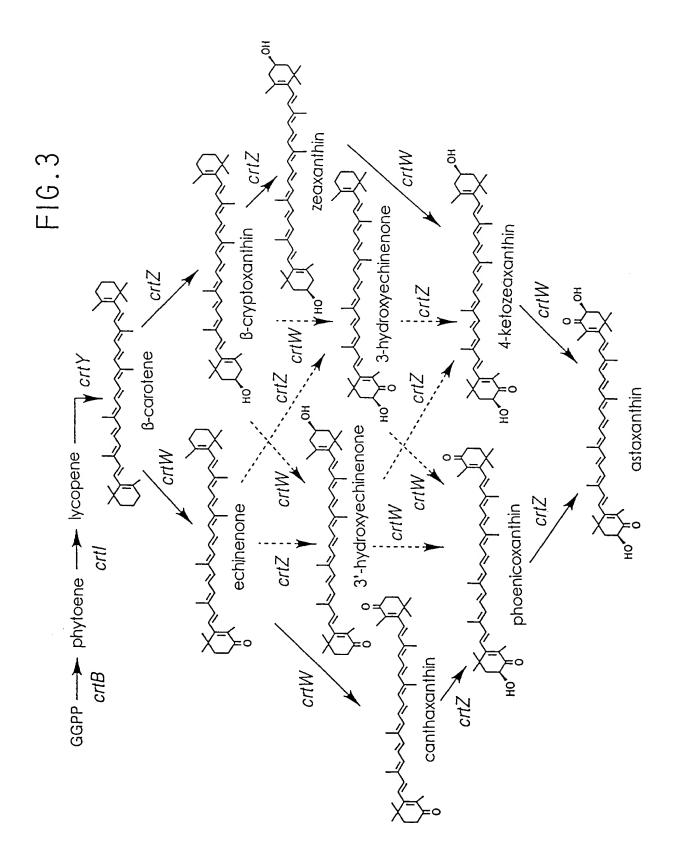
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INCREASING PRODUCTION OF
CAROTENOIDS
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Title: A DNA CHAIN USEFUL FOR INCREASING PRODUCTION OF CAROTENOIDS

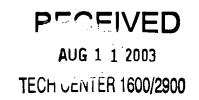
Inventor(s): Susmu KAJIWARA et al. Appl. No.: 08/737,319 AUG 1 1 2003
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<u> </u>						1	1	<i>)</i> .	1								
1		9			18			27			36			45			54
ATG	TCC	ATG	CCC	AAC	ATT	GTT	CCC	CCC	GCC	GAG	GTC	CGA	ACC	GAA	GGA	CTC	AGT
			Pro														
																	18
		63			72			81			90			. 99			108
			TAC														
Leu	Glu	Glu	Tyr	qeA	Glu	Glu	Gln	Val	Arg	Leu	Met	Glu	Glu	Arg	Суз	Ile	
											144			153			36 162
		117	a. a	63.5	126	CCC	m a m	135	CAC	CCT	144 TCC	222	AAG		ምርር	CAC	
			GAC Asp														
val	ASR	PIO	ASP	Asp	Val	ATA	TÄT	GIY	GIU	AL a	267	בעם	232	****	CJ.		54
		171			180			189			198			207			216
ATG	TCC		ATC	AAC		CCC	AAG		CTC	CTC		CGA	GCA		TCC	GTG	TTT
			Ile														
							•	-									72
		225			234			243			252			261			270
			CCA														
Leu	Phe	Arg	Pro	Ser	Asp	Gly	Ala	Leu	Leu	Leu	Gln	Arg	Arg	Ala	qeA	Glu	
																	90
		279			288			297			306			315			324
			CCT														
Ile	Thr	Phe	Pro	GTĀ	Met	Trp	Tnr	ASN	Thr	Суз	Суз	Ser	uis	PLO	Dea	Ser	108
		333			342			351			360			369			378
AAG	ccc		GTT	GAA		GAG	AAC		ATC	GGT		CGA	CGA		GCG	TCC	
			Val														
	2									-							126
		387			396			405			414			423			432
																	TTC
Lys	Leu	Glu	His	Glu	Leu	Gly	Val	Pro	Thr	Ser	Ser	Thr	Pro	Pro	Asp	Ser	Phe
														477			144
		441			450			459		000	468	C1C	CCI	477		CCN	486
			ACT														
Thr	Tyr	Leu	Thr	Arg	TTE	H13	туг	rea	Ala	PIO	Ser	rap	GIY	Deu	rrp	GLY	162
		495			504			513			522			531			540
CAC	GAG		GAC	TAC			TTC			ACA		ACA	GAA			GGA	
			Asp														
				- 4													180
		549			558			567			576			585			594
			GTC														
Pro	Asn	Glu	Val	Ser	Asp	Thr	Arg	Tyr	Val	Thr	Lys	Pro	Glu	Leu	Gln	Ala	
																	198



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		603			612			621			630			639			648
TTT	GAG	GAC	GAG	TCT	AAC	TCA	TTT	ACC	CCT	TGG	TTC	AAG	TTG	ATT	GCC	CGA	GAC
Phe	Glu	Asp	Glu	Ser	Asn	Ser	Phe	Thr	Pro	Trp	Phe	Lys	Leu	Ile	Ala	Arg	qeA
																	216
		657			666			675			684			693			702
TTC	CTG	TTT	GGC	TGG	TGG	GAT	CAA	CTT	CTC	GCC	AGA	CGA	AAT	GAA	AAG	GGT	GAG
Phe	Leu	Phe	Gly	Trp	Trp	Asp	Gln	Leu	Leu	Ala	Arg	Arg	Asn	Glu	Lys	Gly	Glu
																	234
		711			720			729			738			747			756
GTC	GAT	GCC	AAA	TCG	TTG	GAG	GAT	CTC	TCG	GAC	AAC	AAA	GTC	TGG	AAG	ATG	TAG
Val	Asp	Ala	Lys	Ser	Leu	Glu	Asp	Leu	Ser	qeA	Asn	Lys	Val	Trp	Lys	Met	***
																251	
																j	B

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Inventor(s): Susmu KAJIWARA et al. Appl. No.: 08/737,319

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1		٠9			18			27			36			45			54
ATG	CAG	CTG	CTT	GCC	GAG	GAC	CGC	ACA	GAC	CAT	ATG	AGG	GGT	GCA	AGT	ACC	TGG
			Leu														
							_										18
		63			72			81			90			99			108
GCA	GGC	GGG	CAG	TCG	CAG	GAT	GAG	CTG	ATG	CTG	AAG	GAC	GAG	TGC	ATC	TTG	GTG
Ala	Gly	Gly	Gln	Ser	Gln	qeA	Glu	Leu	Met	Leu	Lys	Asp	Glu	Суз	Ile	Leu	Val
																	36
		117			126			135			144			153			162
			GAC														
Asp	Ala	qeA	qeA	Asn	Ile	Thr	Gly	His	Val	Ser	Lys	Leu	Glu	Суз	His	Lys	
																	54
		171			180			189			198			207			216
			CAG														
Leu	Pro	His	Gln	Pro	Ala	GLy	Leu	Leu	His	Arg	Ala	Phe	Ser	Val	Phe	Leu	
		225			224			242			252			261			72
CAC	CIC	225	GGG	CCA	234	CTC	CTC	243	CIC	CCT	252 CCN	CCN	ምር እ	261	እጥር	a Ca	270
			Gly														
vəb	лэр	GIII	GTĀ	ALG	neu	Deu	nea	9111	GIII	ALG	n_a	nry	267	273	176	****	90
		279			288			297			306			315			324
CCC	AGT		TGG	ACC		ACC	TGC		AGC	CAC		CTA	CAT		CAG	ACC	
			Trp														
			•				•	•						-			108
		333			342			351			360			369			378
GAT	GAG	GTG	GAC	CAA	CTA	AGC	CAG	GTG	GCC	GAC	GGC	ACA	GTA	CCT	GGC	GCA	AAG
qeA	Glu	Val	Asp	Gln	Leu	Ser	Gln	Val	Ala	Asp	Gly	Thr	Val	Pro	Gly	Ala	Lys
																	126
		387			396			405			414			423			432
GCT	GCT	GCC	ATC	CGC	AAG	TTG	GAG	CAC	GAG	CTG	GGG	ATA	CCA	GCG	CAC	CAG	CTG
Ala	Ala	Ala	Ile	Arg	Lys	Leu	Glu	His	Glu	Leu	Gly	Ile	Pro	Ala	His	Gln	
																	144
		441			450			459			468			477			486
			GCG														
Pro	Ala	Ser	Ala	Pne	Arg	Pne	Leu	Thr	Arg	Leu	HIS	туг	Cys	Ala	ATA	qea	
		405			504			512			522			531			162 540
CNC		495	GCG	101	504	TC N	CCN	513		ccc		CNC	CAA		CNC	መስር	
			Ala														
GIII	PLO	ALA	ма	1111	GIII	251	Ala	Den	ırp	GIŞ	GIU	nra	GIU	Mec	nsp	IYL	180
		549			558			567			576			585			594
TTA	TTC		CGG	GCC		GTC	ACC			CCC			GAC		GTG	GAC	•
			Arg														
			- 3										•			•	198

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		603			612			621			630			639			648
GTC	AGG	TAC	GTG	ACG	CAG	GAG	GAG	CTG	CGG	CAG	ATG	ATG	CAG	CCG	GAC	AAT	GGG
Val	Arg	Tyr	Val	Thr	Gln	Glu	Glu	Leu	Arg	Gln	Met	Met	Gln	Pro	qeA	Asn	Gly
		_															216
		657			666			675			684			693			702
TTG	CAA	TGG	TCG	CCG	TGG	TTT	CGC	ATC	ATC	GCC	GCG	CGC	TTC	CTT	GAG	CGC	TGG
Leu	Gln	Trp	Ser	Pro	Trp	Phe	Arg	Ile	Ile	Ala	Ala	Arg	Phe	Leu	Glu	Arg	Trp
																	234
		711			720			729			738			747			756
TGG	GCT	GAC	CTA	GAC	GCG	GCC	CTG	AAC	ACT	GAC	AAA	CAC	GAG	GAT	TGG	GGA	ACG
Trp	Ala	Asp	Leu	Asp	Ala	Ala	Leu	neA	Thr	Asp	Lys	His	Glu	Asp	Trp	Gly	Thr
																	252
		765			774		780										
GTG	CAT	CAC	ATC	AAC	GAA	GCG	TGA										
Val	His	His	Ile	Asn	Glu	Ala	***										
						259											
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		9			18			27			36			45			54
ATG	ACT	-	GAC	AAC		AGT	ATG		CAT	GGT		GTA	TCT	AGT	TAC	GCC	AAA
									His								
																	18
		63			72			81			90			99			108
									ATT								
Leu	Val	Gln	Asn	Gln	Thr	Pro	Glu	qeA	Ile	Leu	Glu	Glu	Phe	Pro	Glu	Ile	
					700			125			144			153			36 162
CC.	mm s	117	C) 3	101	126	3 3 70	NCC.	135	TCT	ъст	144	»CG	TCB		GNC	CAA	
									Ser								
PLO	rea	GIII	GIII	ALG	FLO	ASII	1111	nra	361	561	014	****	001			0.20	54
		171			180			189			198			207			216
GGA	GAA		TGT	TTT		GGT	CAT		GAG	GAG	CAA	ATT	AAG	TTA	ATG	AAT	GAA
									Glu								
																	72
		225			234			243			252			261			270
									AAT								
Asn	Cys	Ile	Val	Leu	Asp	Trp	Asp	Asp	Asn	Ala	Ile	Gly	Ala	Gly	Thr	Lys	
								007			206			215			90
		279		1 mc	288		3 mm	297	***	CCM	306	Cms	Cam	315	CCA	mm/C	324
									AAG Lys								
AGT	Cys	птз	nea	Mec	GIU	พวเเ	TIE	GIU	nys	GTĀ	nea	Dea	nro	nry	VTG	rne	108
		333			342			351			360			369			378
GTC	TTT		TTC	AAT		CAA	GGT		TTA	CTT	TTA	CAA	CAA	AGA	GCC	ACT	GAA
									Leu								
																	126
		387			396			405			414			423			432
									AAC								
Lys	Ile	Thr	Phe	Pro	Asp	Leu	Trp	Thr	Asn	Thr	Суз	Cys	Ser	His	Pro	Leu	
		443			450			450			468			477			144 486
እ ጥጥ	Car	441 GAC	GNA	ጥጥ እ	450 GGT	TTG	A A G	459 CCT	AAG	СТА		CAT	MAG		AAG	هود	
									Lys								
110	p		020	202	01,	200	-10	0-7	2,0	200			-1-		-1-	- -1	162
		495			504			513			522			531			540
ATT	ACT		GCG	GTG		AAA	CTA		CAT	GAA		GGT	ATT	CCA	GAA	GAT	GAA.
Ile	Thr	Ala	Ala	Val	Arg	Lys	Leu	Asp	His	Glu	Leu	Gly	Ile	Pro	Glu	qeA	Glu
																	180

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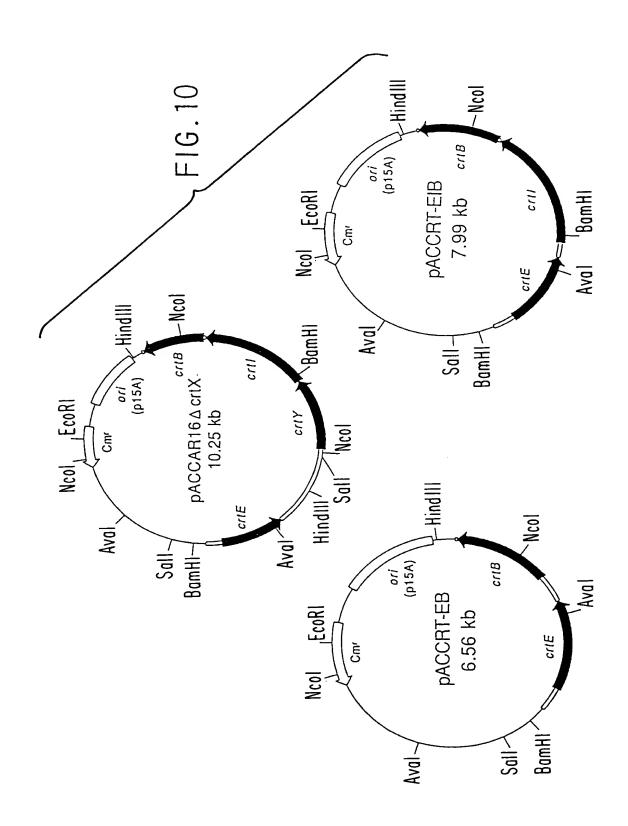
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		549			558			567			576			585			594
ACT	AAG	ACA	AGG	GGT	AAG	TTT	CAC	TTT	TTA	AAC	AGA	ATC	CAT	TAC	ATG	GCA	CCA
					Lys												
	•																198
		603			612			621			630			639			648
AGC	AAT	GAA	CCA	TGG	GGT	GAA	CAT	GAA	ATT	GAT	TAC	ATC	CTA	TTT	TAT	AAG	ATC
					Gly												Ile
				-													216
		657			666			675			684			693			702
AAC	GCT	AAA	GAA	AAC	TTG	ACT	GTC	AAC	CCA	AAC	GTC	AAT	GAA	GTT	AGA	GAC	TTC
Asn	Ala	Lys	Glu	Asn	Leu	Thr	Val	Asn	Pro	Asn	Val	Asn	Glu	Val	Arg	Asp	Phe
		-															234
		711			720			729			738			747			756
AAA	TGG	GTT	TCA	CCA	AAT	GAT	TTG	AAA	ACT	ATG	TTT	GCT	GAC	CCA	AGT	TAC	AAG
Lys	Trp	Val	Ser	Pro	Asn	Asp	Leu	Lys	Thr	Met	Phe	Ala	Asp	Pro	Ser	Tyr	
																	252
		765			774			783			792			801			810
					AAG												
Phe	Thr	Pro	Trp	Phe	Lys	Ile	Ile	Суз	Glu	Asn	Tyr	Leu	Phe	Asn	Trp	Trp	
																	270
		819			828			837			846			855			864
					TCT												
Gln	Leu	Asp	Asp	Leu	Ser	Glu	Val	Glu	Asn	Asp	Arg	Gln	Ile	His	Arg	Met	
																	288
867																	ł
TAA																	F
TUV																	_

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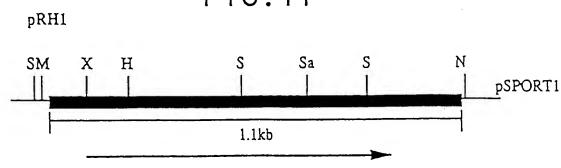


Title: A DNA CHAIN USEFUL FOR INCREASING PRODUCTION OF CAROTENOIDS

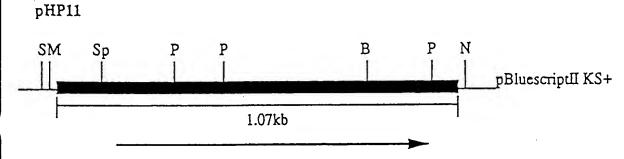
Inventor(s): Susmu KAJIWARA et al.

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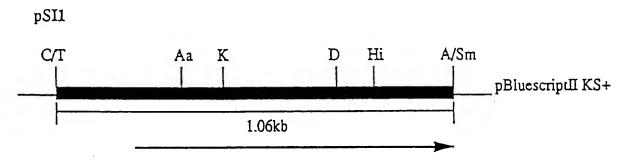




Phaffia rhodozyma IPP isomerase



Haematococcus pluvialis IPP isomerase



Saccharomyces cerevisiae IPP isomerase

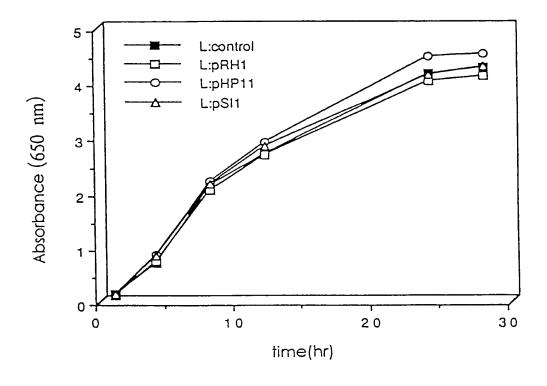
Aa: AatII, A: AccII, B:BssHII, D:DraI, Hi:HincII, H:HpaI, K:KpnI, M:MluI, N:NotI, P:PstI, Sa:SacI, S:SalI, Sp:SphI, X:XbaI



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FIG.12

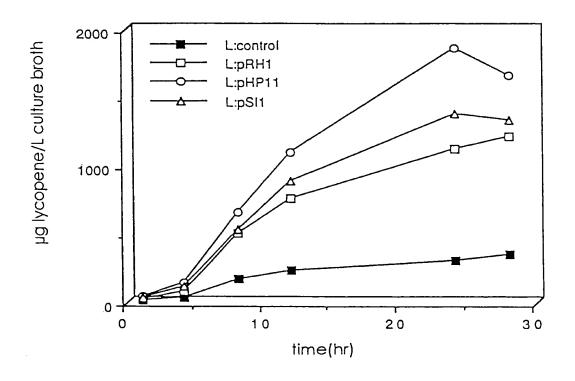




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FIG.13





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E.coli	µg carotene/g dry weight	production
L: control	228	1
L: pRH1	825	3.6
L: pHP11	1029	4.5
L: pSI1	859	3.8
β: control	488	1
β: pRH1	709	1.5
P: control	246	1
P: pRH1	413	1.7
P: pHP11	504	2.1